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BORDEN'S REVIEW of NUTRITION RESEARCH

Summaries and reviews of research information concerning the nutritive values of milk and milk products. Published by The Borden Company, 350 Madison Avenue, New York, N. Y.

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BIOTIN IN NUTRITION*

The early work leading to the discovery of biotin as a vitamin protecting against a nutritional deficiency disease started with the studies of Boas (1) who found that rats maintained on a diet containing uncooked egg-white as the sole source of protein developed a characteristic dermatitis. The work of Boas was followed by that of Lease and Parsons (2) who extended the studies to chicks and found that these animals also developed a dermatitis on a diet rich in raw egg-white. It was not recognized until many years later that the egg-white diet produced this characteristic deficiency disease by indirectly depriving the animal of the vitamin, biotin, through the action of the anti-vitamin, avidin.

Biotin In Animal Nutrition:

Poultry Dermatitis: About eight years after the observations of Lease and Parsons, a similar dermatitis was observed by Hegsted *et al* (3) during studies on the requirements of chicks maintained on highly purified rations. Although the rations did not contain uncooked egg-white, the chicks developed lesions very similar to those seen in egg-white "toxicity". The bottoms of the feet became rough and calloused, later becoming entirely encrusted and exhibiting numerous hemorrhagic cracks. The toes frequently became necrotic and were sloughed off. The top of the feet and legs, however, merely developed a dry scaliness. As the disease progressed the corners of the mouth showed characteristic lesions which soon spread to the area around the beak. In some cases the eyelids became swollen and pasted together. The symptoms were not cured by the known chick anti-dermatitis factor, pantothenic acid.

In view of the fact that the new dermatitis so closely resembled the chick dermatitis brought about by feeding large amounts of raw egg-white, Hegsted and his associates suggested that this dermatitis and the egg-white dermatitis might both be identical with egg-white injury in rats. They administered a wide variety of supplements in an attempt to find a curative factor for the disease. As a result, they found that the curative factor occurred in the same foods as did the anti-egg-white injury factor, vitamin H. Their further studies on the occurrence and chemical properties of the unknown factor pointed very strongly to its identity with vitamin H.

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*The first section of this paper appeared in the February issue of *Borden's Review of Nutrition Research*.

In similar work the following year, Ansbacher and Landy (4) found that chicks maintained on a ration supplying less than 0.1 microgram of biotin (which had now been identified with vitamin H) developed the characteristic dermatitis in 5 to 14 days. They were able to cure the disease by the administration of 1.25 micrograms of crystalline biotin per day. More severe cases responded to a daily dose of 2.5 micrograms. This work clearly indicates the vitamin nature of biotin, in that a direct deficiency disease had been induced by a diet low in this substance (without necessitating the use of avidin) and that subsequently cures were obtained by the specific administration of the pure missing factor.

The total minimum curative dose of biotin was suggested by Ansbacher and Landy to be about 10 micrograms per chick. Hegsted *et al* (5) in a continuation of their earlier work, set the biotin requirement of the chick at approximately 7 to 10 micrograms per 100 grams of purified diet.

These studies demonstrated that chicks develop a true biotin deficiency if the ration is low in biotin. Rats, on the other hand, require the presence of avidin in the diet before deficiency symptoms appear. This would seem to indicate that chicks cannot synthesize biotin. (Incidentally, bacterial synthesis of biotin in the organism can be inhibited in mammals by the incorporation of sulfonamides in the diet (6).)

Coincident with the studies on biotin in chick nutrition, evidence for the essentiality of this vitamin in the nutrition of turkeys was presented by Patrick, Boucher, Dutcher and Knandel (7). They discovered a dermatitis in baby turkeys whose symptoms paralleled almost identically those described by Hegsted in chicks. They found, likewise, after extensive experimentation, that the dermatitis was prevented or cured by biotin-rich foods or by biotin concentrates.

Perosis: These studies on the new dermatitis of chicks and turkey poults established biotin as an essential constituent in their diet. As a result of these observations, the study of biotin in poultry nutrition was stimulated and new researches into other phases of its activity were initiated. In this way it was found that biotin, in addition to preventing poultry dermatitis, plays an important part in the prevention of perosis or "slipped tendon" in chicks and turkeys. Perosis is characterized by the inability of the animals to stand for any length of time and an abnormal gait indicating that walking is painful. This is followed by enlargement and turning in of the hocks and turning out of one foot. In severe cases the tendon of Achilles slips out of place, sometimes in both legs.

Perosis on a low biotin diet was observed as early as 1940 by Ansbacher (8) who reported that chicks receiving a ration containing less than 0.1 microgram of biotin per gram of diet invariably developed the disease within 2 or 3 months. Jukes and Bird (9) reported, two years later, that on a diet containing 30 cc. of fresh raw egg-white per 100 grams of ration from 50 to 70 percent of the chicks developed perosis in 3 to 5 weeks. The perosis, as well as the accompanying dermatitis, was prevented by heating or omitting the egg-

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white or by injection of as little as 0.34 microgram of crystalline biotin per chick each day. Jukes and Bird found, however, that this low dose of biotin while effective in completely curing the perosis, did not entirely cure the dermatitis, as indicated in the following table:

Supplement	*Birds Showing* Perosis %	Dermatitis %	*Average Wt.* Grams
None	55	82	216
Biotin	0	38	285

Somewhat different results regarding the biotin requirement in poultry dermatitis and perosis were reported by Richardson, Hogan and Miller (10) at the Missouri Agricultural Experiment Station. They found that on an egg-white and sucrose ration chicks developed perosis first (in 8 to 14 days) and dermatitis later (not until about 28 days). Furthermore, administration of from 2 - 5 micrograms of biotin per chick each day completely protected them from the dermatitis, but not from the perosis.

It has been known for many years that factors other than biotin, such as choline and manganese, are involved in the prevention of perosis in chicks. Richardson *et al* suggest the existence of still another anti-perotic factor which they have derived from a specific type of beef liver extract. From their observations that biotin cures chick dermatitis but not perosis, they conclude that there may be an additional factor besides biotin, manganese, choline and a liver extract factor, concerned in the prevention of perosis. If there is not such a fifth factor, they suggest the alternative possibility that the anti-perotic liver extract derivative may be inactivated by a constituent of egg-white which, however, does not seem to be avidin.

From these rather complex data, the conclusions at present seem to be that:

(1) Biotin is required to prevent a special type of dermatitis in chicks and turkeys, and

(2) Biotin is one of the factors in the antiperosis complex required to prevent perosis in chicks and turkeys.

Avian Reproduction: In another field of poultry research, a group of investigators, consisting of Cravens, Sebesta, Halpin and Hart (11) were attempting to develop suitable purified rations to study the effect of various B vitamins on egg production and hatchability. During the course of this work they observed a marked fall in the hatchability of eggs produced by hens on a ration low in biotin. There was a decrease in hatchability from 80% of the fertile eggs to less than 20% in six weeks, but no noticeable effect on actual egg production. After considerable experimentation, they found the missing dietary factor could be supplied by *whey concentrate,* by *kidney residue* or by *biotin.* Supplementing the diet with any of these raised the level of hatchability from about 10% to at least 80% in three weeks. On the low-hatchability diet, no dermatitis was noted, so that it was assumed a bare minimum of biotin was

present in the basal ration. Because of the present high cost of pure biotin, the authors recommend the use of whey concentrate which is rich in biotin as an effective supplement to rations on which low hatchability is observed.

Another interesting paper indicates that avidin, as well as biotin, may be related to reproduction in the fowl. Hertz and Sebrell (12) at the National Institute of Health presented evidence that avidin arises as a secretory product in the oviducts of birds. (12) Early this year Fraps, Hertz and Sebrell (13) reported that the avidin-biotin complex may be intimately involved in the physiology of poultry reproduction. They reached this conclusion from a study of the tissues where avidin occurrence is highest; from comparisons between avidin secretions of laying and non-laying hens, and from direct observation on the experimental stimulation of avidin secretion by sex hormones administered to immature chicks and non-laying hens (14).

Biotin In Human Nutrition:

"Egg-white Injury" in Man: In view of the growing significance of biotin in animal nutrition, it seemed important to determine whether any of the phenomena of human deficiency disease might be related to biotin. That biotin might play a significant role in human nutrition was indicated as far back as 1929 by Findlay and Stern (15) who noted a marked resemblance between "pink disease" in children and the symptoms described for "egg-white injury" in rats. Although Findlay and Stern suggested the possibility of a deficiency disease in relation to the egg-white injury factor, no further work along these lines was pursued and therefore no definite conclusion was reached at that time. In 1942, a specific experiment on human beings was conducted by Sydenstricker, de Vaughn, Singall, Briggs, de Vaugh and Isbell (16) in the hope of determining the relation, if any, of biotin to human nutrition.

For this purpose, a small group of adults volunteered to eat a special diet designed to contain a minimum of biotin. To this low biotin diet was added 200 grams of dehydrated egg-white which constituted somewhat more than 30 per cent of the total daily caloric intake. Because the diet was poor in minerals and in vitamins of the B complex, except riboflavin, sufficient vitamin and mineral supplements were given to correct these deficiencies. Four of the volunteers continued on the experiment until satisfactory conclusions were obtained. Typical vitamin deficiency symptoms developed in a few weeks in all of the subjects. Most of the symptoms were rapidly cleared up in 3 to 5 days by the administration of biotin concentrate in doses ranging from 150 to 300 micrograms of biotin a day. The investigators conclude that biotin deficiency leads to a definite avitaminosis syndrome in man.

Skin Disorders: The relation of vitamins to various skin disorders has been hinted at for many years, but the nutritional approach to experimental dermatologic research has, as yet, not received thorough consideration. The lesions

arising in the condition of pellagra, of course, are well recognized as a skin disorder resulting from a nutritional deficiency. This is also true for certain other skin lesions, as for example those caused by lack of vitamin A (17). Beyond these instances, not much has been reported.

In a preliminary paper published in 1941, György (18) presented a number of interesting facts on the relation of diet to the treatment of certain classes of dermatoses. He suggested that egg-white injury in rats might be related to a dermatitis in man which is characterized by scaliness and desquamation of the skin and by excessive activity of the sebaceous glands. He set forth in detail the well-known symptoms of egg-white injury in rats and compared them with the symptoms of certain human skin disorders including such infant eczemas as intertrigo, "cradle cap" and Leiner's disease.

From his observations on the causes and characteristics of animal skin disorders brought about by deficiencies in biotin, as well as by other B vitamins such as riboflavin, pyridoxine and pantothenic acid and by comparison of the animal symptoms with various seborrheic disorders in man, György concludes that these vitamins might very well play an important part in the cause and cure of such human dermatoses. He points out that although liver and yeast are good sources of these vitamins, the amounts of these to be eaten daily would be too high to be practicable. According to György:

> **"Only one really common foodstuff, cow's milk, is rich in both pyridoxine and riboflavin and also contains appreciable amounts of biotin. The fact that the content of biotin in human milk is low could serve to corroborate . . . the higher incidence of Leiner's disease in breast-fed infants."**

Biotin and Cancer: Many compounds, from chemical dyes to various food constituents, have at one time or another been tested in connection with the study of cancer in the hope of finding a substance which would either be peculiar to cancer or perhaps occur more prominently in cancerous than in normal tissues. Recently, in this connection, interest has been focussed on biotin.

During the course of an investigation of the biotin content of various types of tissues, West and Woglom (19, 20) of the Department of Cancer Research at Columbia University, reported some interesting data. They found, first of all, that certain cancer or tumor tissues contained much more, and others significantly less biotin than did normal adult tissues. A second observation was that embryo tissues deviated from the normal biotin levels in the same manner as the corresponding tumor tissues. Subsequently, they attempted to alter the biotin content of tumors experimentally and thus to influence their growth. To this end, they removed certain types of tumors from test animals and transplanted these tumors to animals dying of avidin-induced biotin deficiency. Strangely enough, although the host animal succumbed to the lack of biotin, the transplanted tumors continued to grow and flourish, despite the lack of biotin. Because biotin is indispensable to many microorganisms and higher animals it was thought that the usual deviations in biotin content of

embryo and tumor tissue might reflect fundamental changes in metabolism peculiar to rapidly growing cells. This possibility, however, was not substantiated by the results of the above-described experiments. In fact, from the unimpaired growth of the transplanted tumors, West and Woglom conclude that:

> "While biotin is known to be an essential factor in the metabolism of normal mammalian tissues, these tumors, at least, are capable of maintaining their usual activity in its relative absence."

Pollack, Taylor, Taylor and Williams in Texas conducted similar investigations, extending their studies to the variations in riboflavin (21), nicotinic acid (22) and pantothenic acid (23) contents of normal and tumor tissues as well as to the biotin variations (24). From their findings, cancer tissues were not indicated as especially biotin-rich nor as requiring excess biotin for growth. While the biotin content of tumor tissues differs from that of normal tissues, the variations may be a result of the cancerous state than the cause of it.

Before the publication of all these studies, an interesting hypothesis was set forth by Laurence (25) on the possible relation of biotin deficiency to spontaneous recessions in cancer. Spontaneous recoveries are not uncommon in the history of cancer, although their causes are not understood. From cases cited in medical literature indicating that many such recessions occurred during or following acute infectious diseases and from the preliminary indications of West and Woglom that many tumors are richer in biotin than normal tissues, Laurence postulated that both malignant cells and the microorganisms causing infectious diseases require excess biotin. On this assumption, Laurence suggested that in infectious diseases, the microorganisms causing the disease used up all the biotin thereby depriving the cancer cells of the vitamin and checking their growth, thus explaining the occurrence of a spontaneous recession. Laurence went on further to suggest that if the foregoing hypothesis were true, it might be possible to control tumor growth by the administration of avidin, the biotin-binding factor, which would, like the germ microorganisms, presumably deprive the tumor of required biotin. There are certainly very attractive possibilities in such a theory; however it must be pointed out that:

> (1) The presence of large amounts of biotin in certain tumors does not necessarily imply an increased biotin requirement of these tissues; conceivably it may be that precisely the converse is true — that the presence of large amounts of biotin indicate it is not being utilized by the tissue.
>
> (2) Later studies by West and Woglom and by Williams *et al* show no apparent relation between biotin requirement and tumor growth, some tumors being high, others low in biotin and still others growing independently of biotin supply, while normal tissues under such circumstances were unable to live.

At the present time, therefore, there is no scientific indication that cancer cells specifically require biotin. Despite this fact, investigators are maintaining an open mind to all possibilities however remote and, for this reason, have been and still are investigating the possibilities of avidin administration in the control of abnormal cell growth. Recently, Rhoads and Abels (26) at the Memorial Hospital in New York City reported on the effect of clinical administration

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of uncooked egg-white and avidin concentrates to two cancer patients on a low biotin diet. They administered, over a period of thirty weeks, from 16 to 40 times the amounts of avidin necessary to bind whatever biotin was present in the diet. During this time they did not note any clinical evidence of biotin deficiency as had been reported by Sydenstricker *et al,* nor did they find any significant decrease in the urinary excretion of biotin. There was no effect at all on the course of the cancers. From this study it is apparent that the avidin-biotin complex has no discernable influence upon human cancers of the type studied.

Another aspect of biotin activity in relation to experimental cancer has recently aroused much interest. The story goes back to the discovery, about 1937, by Japanese investigators that certain coal tar dyes are capable of causing a special type of liver cancer when fed to rats. Since that time a great deal of study has been devoted to the carcinogenic action of these dyes, most of the work having been with the dye known as *p-dimethylaminoazobenzene*. This dye, which is popularly known as "butter yellow," is *not* employed in this country as a coloring agent. Its use is not permitted under the Federal Food law of 1906 or the more recent Federal Food, Drug and Cosmetic Act of 1938. Because this dye causes an experimental type of liver cancer, it was thought that studies in this field might throw some light on causes of other types of cancer. To date, several interesting facts have been revealed from the study of artificial cancer induced by p-dimethylaminoazobenzene. These are:

1. The dye, p-dimethylaminoazobene, induces cancer in rats on an experimental diet of brown rice and carrots.
2. When riboflavin and the milk protein casein were added to the diet of brown rice, carrots and p-dimethylaminoazobene, 96% of the rats were protected from the cancer-stimulating effect of the dye (27).
3. When other B vitamins were added to the protective riboflavin-casein diet, no greater protection against the dye was afforded than with riboflavin and casein alone (28).
4. When biotin was added to the protective riboflavin-casein diet, about half of the protection was lost (28).

These studies indicate that biotin, for some unknown reason, interferes with the protective action of riboflavin and casein in the prevention of this type of experimental liver cancer. The significance of these experiments cannot be interpreted until the results of further studies become available; nor can any conclusions be drawn from them regarding human cancer. It is pointed out by some investigators that not only casein, but methionine and choline exert a protective influence against "butter yellow" cancer. A detailed report on this phase of the research has not yet appeared; when it does, it will be interesting to learn whether the labile methyl groups which these compounds all have in common (cf. *Borden's Review of Nutrition Research,* Oct. 1942) are in any way connected with the protective mechanism.

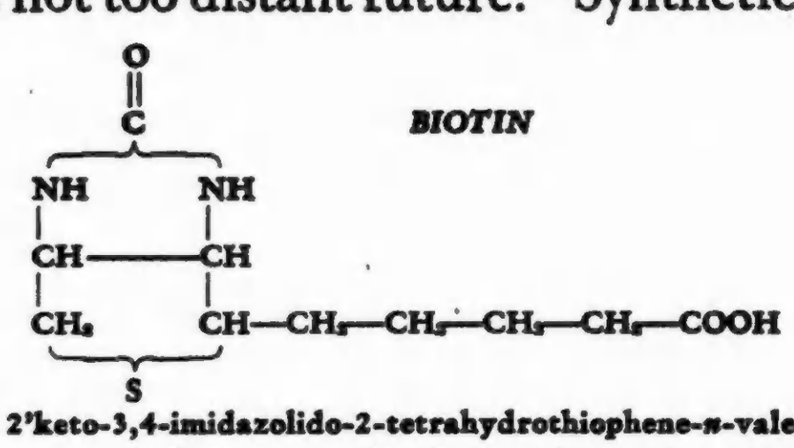
Biotin and Malaria: In February, 1943, a preliminary note was published by Trager (29) at the Rockefeller Institute for Medical Research in which he

reported that susceptibility to malaria in chickens and ducks is greatly influenced by the level of biotin in the tissues of the animal. He found that biotin-deficient fowl were more susceptible to malaria and showed a greater incidence of mortality than did normal control animals. Administration of biotin to the deficient animals lessened the severity of the malarial infection. The applicability of these results to man must necessarily await extended observations correlating body biotin levels in man with the degree of susceptibility to malaria before any conclusions may be drawn. We can only speculate as to whether the information will prove of value in the present critical situation confronting our fighting forces in tropic areas where malaria is prevalent.

The Human Requirement for Biotin: The biotin requirement for man has not yet been established, but an estimated figure has been suggested by Williams (32). This figure was derived by using the biotin content of cow's milk as a working basis. Cow's milk contains approximately 0.06 milligram biotin per 100 calories, a presumably adequate ratio. Accordingly, a 2500-calorie average daily intake for adults would establish, on the same ratio basis, a hypothetical biotin requirement of 0.15 milligram per day.

The Future Of Biotin Research:

Only a few months ago, the completion of a brilliant series of researches at Cornell University (30) revealed the exact chemical nature and formula of this new vitamin thereby opening up the possibility for laboratory synthesis of biotin in the not too distant future.* Synthetic biotin will give great impetus to research in the biology of this vitamin. Such research is limited at present, because biotin is still very expensive, although recently a less expensive method has been developed for obtaining greater yields of biotin from milk (31) than have heretofore been possible from liver or egg yolk.



BIOTIN

2'keto-3,4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid

The story of biotin has merely begun. So far, it has been learned that biotin is essential for all types of living tissue and appears to be involved in many types of metabolic processes. Biotin is required to prevent a characteristic dermatitis in poultry and is also an anti-perosis factor. It is possible that the near future will reveal more definite indications of its activity in the nutrition and physiology of man.

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*As this paper goes to press, workers from the Research Laboratories of Merck & Co., Inc., have announced that biotin has been obtained by a total synthesis and has been found to be identical with the natural product (33). This synthesis verifies the structure assigned to biotin by duVigneaud and co-workers (30).

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NEWS DIGEST

HIGH PROTEIN DIETS have been shown experimentally to hasten the repair of wounds whether caused by operation or otherwise. This is because the loss of body nitrogen is relatively high during the first few days after the infliction of the wound. A high protein diet is therefore indicated to replace this loss. Accordingly, it is recommended that milk, grated cheese, and eggs be included in the diet from the earliest days.

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THIAMIN AND RIBOFLAVIN, when fed to cows, according to reports from abroad, resulted in a 10% increase of total available butterfat. It was reported that the feeding of 5 mg. of live baker's yeast or of a combination of 50 mg. thiamin and 6 mg. riboflavin per cow each day resulted in a rise in the fat content of the milk from a level of 4% to a level of 5.3%. During the winter months thiamin and riboflavin were administered in larger doses amounting to 60-200 mg. thiamin and 20 mg. riboflavin. The total milk production remained constant.

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CHEDDAR CHEESE is the richest source of riboflavin available from common foods purchased on the retail market in New York City. The riboflavin values range from 546 to 600 micrograms per gram of cheese. Milk, beef muscle and salmon are also rich sources, each having over 200 micrograms of riboflavin per gram. Other good sources among available market foods include dried legumes, fresh peas and green endive.

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YEAST TABLETS providing B complex vitamins, have been perfected for use by consumers as an alternative to fresh yeast cakes. Two tablets are said to be the equivalent in thiamin, riboflavin and niacin value of one yeast cake. The tablets cannot be used as a leavening agent for baking.

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FROZEN CHICKEN A LA KING is the newest addition to the frozen food family. A typical product consists of light and dark chicken, sliced mushrooms, green peppers, pimento and other seasonings, together with a chicken broth in a rich, creamy gravy. Preparatory to serving, milk should be added and the mixture heated. The regular package serves from two to three.

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"DRY MILK POWDER", according to a noted nutrition authority, "is a munition of war." With the present critical food situation, a serious protein shortage is being encountered. Decreasing supplies of fish, meat and eggs throw the burden of the proteins supply upon cereals, which are plentiful and inexpensive. With the use of milk, either as a supplemental food or as a component of bread flours, incomplete cereal proteins can be made nutritionally adequate and can serve as a source of protein to the extent of as much as 40% of the diet. The desired goal is the incorporation of 6% milk solids in bread, thus assuring universal nutritional protection. It has been stated that we can live adequately for a long time and obtain all nutritional essentials on a diet of bread enriched with 6% milk solids and a suitable source of vitamin C.

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BANANA POWDER combined with dextrose and powdered skim milk forms the base of a newly introduced product. Banana powder alone was formerly available only to the baking and confectionery trades and to physicians for use in infant feeding, but the new product is now generally available in the retail market and should find many desirable uses.

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DEHYDRATED EGGS retain practically all of the vitamin A content of fresh eggs, according to recent spectroscopic tests. Comparisons between fresh and dried eggs showed less than 5% loss of vitamin A during drying; while losses after storage at 18° C. for 14 weeks were the same for both fresh and dried eggs. The results of spectrographic analyses were confirmed by biological assay tests.

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For further information on any of the above items address
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